TWO NEW SPECIES OF *TRICHOGRAMMA* (HYMENOPTERA: TRICHOGRAMMATIDAE) FROM THE RYUKYU ISLANDS, JAPAN

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Abstract.—Two new species of Trichogramma Westwood from the Ryukyu Islands were collected in green pepper fields using trap cards containing *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and from parasitized *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae) eggs. ITS-2 sequences and scanning electron micrographs were obtained for both *Trichogramma* species. Herein we describe *Trichogramma cultellus* n. sp., and *T. umerus*, n. sp.

Key Words: Trichogramma, new species, Ryukyu Islands

Species of Trichogramma Westwood (Hymenoptera: Trichogrammatidae) in the Ryukyu Islands are poorly known. Ishii (1938, 1941) first recorded T. chilonis Ishii as an egg parasitoid of Tetramoera (= Eucosma) schistaceana (Snellen) (Lepidoptera: Totricidae) from Okinawa. Since then, there have been no records of additional species of Trichogramma from the Ryukyu Islands. In 1997, members of the Shimane Agricultural Experiment Station collected Trichogramma from trap Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) egg cards and from parasitized Plutella xylostella (Linnaeus) (Lepidoptera: Yponomeutidae) eggs in Ishigaki and Iriomote Islands, respectively. Unique ITS-2 DNA sequences and microscopic examination indicated two taxa that have not been previously described. Herein we describe two new species from the Ryukyu Islands, Japan.

MATERIALS AND METHODS

Species descriptions and specimens prepared for scanning electron microscopy (SEM) and DNA analysis are based on methods described previously (Taylor et al., in press). Species descriptions and measurements follow the anatomical terminology, morphological measurements, and ratios (relative dimensions) used in Pinto (1999). All types are deposited in the Entomological Laboratory, Faculty of Agriculture, Kyushu University (ELKU).

Trichogramma cultellus Jose, Hirose, and Honda, new species (Figs. 1–6)

Diagnosis.—*Trichogramma cultellus* is similar to the following species: *T. thalense* Pinto and Oatman, *T. parkeri* Nagarkatti, *T. pintoi* Voegele, and *T. elegantum* Sorokina.



Figs. 1–3. *Trichogramma cultellus*, male. 1, Dorsal view of genital capsule; scale bar = $50 \ \mu\text{m}$. 2, Ventral view of genital capsule (IVP partially broken in preparation); scale bar = $50 \ \mu\text{m}$. 3, Ventral view of apical distance (IVP partially broken in preparation); scale bar = $30 \ \mu\text{m}$.

The female ovipositor length is much shorter in these species measuring 0.79, 0.86-0.95, 0.84, and $0.95 \times$ the hind tibia length for T. thalense, T. parkeri, T. pintoi, and T. elegantum, respectively, vs. 1.04-1.07 in T. cultellus. The genital capsule is generally broader in T. thalense with a width to length ratio greater than 0.30. Ventral processes are also positioned at the base of the intervolsellar process (IVP). In contrast, T. cultellus has a genital capsule width to length ratio of about 0.30 and ventral processes located distinctly anterior to the base of the IVP. Flagella setae are much longer in T. cultellus measuring over $3.5 \times$ the flagellum width vs. T. pintoi (2.56×), T. parkeri (2.85 \times), and T. elegantum (2.2 \times). The posterior extension of the dorsal lamina is much narrower in T. cultellus and T. parkeri than in T. pintoi. However, the dorsal lamina is only $1.5 \times$ as long as wide in T. cultellus but over twice as long as wide in T. parkeri.

Description.—Quantitative data taken from 7 males with a hind tibial length (HTL) of 0.14-0.16 mm and 2 females with HTL = 0.14-0.15 mm unless otherwise indicated. Color recorded from dried specimens wrapped in tissue paper. Both sexes with extensive brown suffusion on metasoma and a dull yellow to tan mesosoma.

Forewing relatively narrow, 0.25 ± 0.01 mm wide (n = 5); width (FWW) 0.52 ± 0.02 length (FWL) (n = 5); setation relatively sparse, 7–13 (n = 4) setae between 4th and 5th setal tracks; longest fringe setae 0.13 ± 0.03 (n = 5) FWW, approximately $1.5-2.1 \times$ hind tibial width (HTW). Hind wing with 0 (n = 2) and 3–5 (n = 2) setae in anterior and posterior tracks, respectively, the latter tract occupying 35–36% distance from hamuli to wing apex. Scutellum with anterior pair of setae 91–95% (n = 2) length of posterior pair.

Male: Flagellum length (FL) 0.16–0.19 mm, approximately $2.4 \times$ longer than scape, FL 6.57 \pm 0.63 \times greater than flagellum width (FW), FL 1.22 \pm 0.11 \times greater than HTL; flagelliform setae long, tapering toward apex, longest flagelliform setae $3.53 \pm 0.57 \times$ greater than FW; without unsocketed setae; basiconic peg sensilla (BPS) relatively small, slightly inflated, formula 2-2-2-1-1-1 (n = 6); terminal placoid sensilla (PLS) with apical 0.23–0.34 extending beyond flagellum apex.

Genital capsule (GC) relatively narrow, 0.30 ± 0.01 as wide as long; apical distance (AD) 0.19 ± 0.01 entire genital length (GL); apical width (AW) 0.68 ± 0.04 genital width (GW); dorsal aperture length (DAL) 0.49 ± 0.02 GL; dorsal lamina (DLA) arising in anterior half of GC without approaching GC sides, slender posterior DLA extension narrows and sharply tapers toward apex occupying 0-0.42 AD (see Variation), width at level of intervolsellar process (IVP) less than that of aedeagus; DLA 1.68 \pm 0.34 (see Variation) as long as wide and 0.47 \pm 0.04 GL; IVP short, subtriangular, occupying 0.11-0.17 AD; volsellae (VS) relatively straight, occupying 0.42-0.61 AD; ventral ridge (VR) narrow and abruptly widening anteriorly, occupying 0.45-0.58 basal distance (BD); ventral processes (VP) not obviously protuberant, slightly laterally displaced to VR and positioned distinctly anterior to IVP. Aedeagus length (AL) ca. equal to GL, 0.76 ± 0.07 (n = 6) HTL; apodemes occupying ca. 0.58 AL.

Female: Antennal funicle with 1 BPS on first funicular segment (F1) and 2 on second (F2). Ovipositor length (OL) $1.04-1.07 \times$ that of HTL.

Types.—Holotype δ : JAPAN. Okinawa Prefecture: Iriomote Island; 4 November 1997; *E. kuehniella* trap host; Y. Narai and N. Itagaki. Paratypes: 6 δ , 19 including allotype \Im , same data as holotype.

Etymology.—Derived from the Latin word *cultellus* meaning small knife, referring to the knife like appearance of the posterior extension of the DLA, used as a noun in apposition.

Variation.—The posterior extension of the DLA usually occupies 0.22–0.42 of the



Figs. 4–6. *Trichogramma cultellus.* 4, Dorsal and ventral view of male genital capsule. 5, Forewing. 6, Hindwing. Scale bars = $0.05 \mu m$.

AD, although in one of the paratypes it fails to reach the level of the AD.

Remarks.—*Trichogramma cultellus* keys to couplet 23' in Pinto's (1999) key to the North American *Trichogramma* but is separated from *T. parkeri* by characters discussed in the Diagnosis section.

DNA sequence.—The 431 bp ITS-2 DNA sequence has been deposited in the NCBI Database with the following accession number: AY518693. We compared this sequence with sequences of species included in the Parkeri section to which *T. cultellus* belongs. A 581 bp *T. pintoi* (accession AY182757) and a 556 bp *T. bourarachae* Pintureau and Babault sequence (accession AF043626) was found to be only 39% and 46% similar to *T. cultellus*.

Trichogramma umerus Jose, Hirose, and Honda, new species (Figs.7–13)

Diagnosis.—*Trichogramma umerus* most closely resembles *T. pretiosum* Riley and *T. minutum* Riley. Females of the latter species have a longer ovipositor, measuring 0.99 and $1.14\times$ the hind tibial length, respectively, vs. 0.85 for *T. umerus*. The *T. minutum* male is most easily differentiated from *T. umerus* in that the latter has longer flagellar setae, a shorter ventral ridge, and a lesser extension of the terminal placoid sensilla on the flagellum. From *T. pretiosum*, *T. umerus* is most easily distinguished by having broader shoulders on the dorsal lamina. The dorsal lamina width to length ratios measure 1.38 and 1.67 for *T. umerus* and *T. pretiosum*, respectively.

Description.—Quantitative data from 8 males with a hind tibial length (HTL) of 0.13-0.16 mm and 3 females with HTL = 0.14-0.17 mm, unless otherwise indicated. Both sexes apparently yellow with brown suffusion on mesosoma and an entirely brown metasoma.

Forewing narrow, 0.21 ± 0.02 (n = 7) mm wide; width (FWW) 0.47 ± 0.03 (n = 7) length (FWL); 9–21 setae between 4th and 5th setal tracts (n = 5); longest fringe setae 0.19 ± 0.02 (n = 7) FWW, ca. $2.38 \times$ greater than hind tibial width (HTW) (n = 7). Hind wing with 1–3 and 4–8 setae in anterior and posterior tracts, respectively, latter tract occupying approximately 52% of



Figs. 7–9. *Trichogramma umerus*, male. 7, Dorsal view of genital capsule; scale bar = 50 μ m. 8, Ventral view of genital capsule; scale bar = 50 μ m. 9, Ventral view of apical distance; scale bar = 20 μ m.



Figs. 10–13. *Trichogramma umerus.* 10, Dorsal and ventral view of male genital capsule. 11, Forewing. 12, Antenna. 13, Hindwing. Scale bars = $0.05 \mu m$.

distance from hamuli to wing apex. Scutellum with anterior pair of setae measuring 86-91% posterior pair (n = 2).

Male: Flagellum length (FL) 0.15-0.18 mm, $1.92-2.42 \times$ longer than scape, FL $6.85 \pm 0.44 \times$ greater than flagellum width (FW), FL $1.16 \pm 0.09 \times$ HTL; flagelliform setae long, $3.0 \pm 0.12 \times$ greater than FW; without unsocketed setae; basiconic peg sensilla (BPS) relatively small, slightly inflated, formula 1(2)-2-2-0-1-1, terminal placoid sensilla (PLS) with apical 0.09-0.16 (n = 6) extending beyond flagellum apex.

Genital capsule (GC) 0.33 ± 0.01 as wide as long; sides not constricted at level of intervolsellar process (IVP); parameres (PM) relatively straight, slightly convergent at apex; apical distance (AD) measuring

 0.26 ± 0.01 entire genital length (GL); apical width (AW) 0.60 ± 0.03 genital width (GW); dorsal aperture length (DAL) $0.56 \pm$ 0.02 GL; dorsal lamina (DLA) originating posterior to middle of GL, moderately notched at base with moderately developed shoulders, not reaching sides of GC; sides of DLA gradually narrowing posteriorly to form a sublinguiform posterior extension, distinctly acuminate at apex, with width approximately equal to that of aedeagus at intervolsellar process (IVP) level; DLA 1.38 \pm 0.05 as long as wide 0.39 \pm 0.02 GL, occupying 0.42-0.57 AD; volsellae (VS) slightly bowed occupying 0.42-0.57 AD; IVP elongate, narrowing apically toward pointed apex, occupying 0.30-0.43 AD; ventral processes (VP) not obviously protuberant, positioned immediately basal to

IVP; VR occupying 0.24–0.34 basal distance (BD). Aedeagus length (AL) approximately equal to GL, attaining 0.77 \pm 0.05 HTL; apodemes about 0.49 AL.

Female: Antenna with 1 and 2 BPS on first funicular segment (F1) and second funicular segment (F2), respectively. Ovipositor length (OL) 0.82–0.85 HTL.

Types.—Holotype δ : JAPAN. Okinawa Prefecture: Ishigaki Island; 13 March 1997; *P. xylostella*; Y. Narai and N. Kajitani. Paratypes: 7 δ and 3 \mathfrak{P} including allotype \mathfrak{P} , same data as holotype.

Etymology.—Derived from the Latin word *umerus* meaning shoulder, referring to the distinct shoulders of the DLA present in the type series, used as a noun in apposition.

Remarks.—*Trichogramma umerus* keys to couplet 74' in Pinto's (1999) key to the North American *Trichogramma* which terminates with *T. pretiosum*. These species are remarkably similar and even share the characteristic of having a poorly sclerotized posterior extension of the dorsal lamina. However, *T. umerus* has a much different ITS-2 sequence (see below) and these species can be separated by characters discussed in the Diagnosis section.

DNA sequence.—The 526 bp ITS-2 DNA sequence for *T. umerus* has been deposited in the NCBI Database (accession AY518694). We performed pair-wise comparisons with both *T. minutum* (NCBI #AF408658) and *T. pretiosum* (NCBI #AF082822) to determine their sequence compatibility. The *T. minutum* 420 bp sequence was 59% compatible to that of *T. umerus* while 400 bp *T. pretiosum* sequence was 53% compatible. Interestingly, in *T. minutum* and *T. pretiosum*, there was a high degree of compatibility at the very beginning and middle of the sequence alignment although a few extra tandem repeats were found in the *T. umerus* sequence. In this section there appeared to be higher compatibility in *T. minutum* than in *T. pretiosum* as more base pairs were shared. However, the final one-third sequence alignment could not be matched in either *T. minutum* or *T. pretiosum* as there was a large extension of ca. 100 base pairs in *T. umerus*.

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